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(54) Title: USE OF HYPOTHALMIC INHIBITORY FACTOR IN THE TREATMENT OF ISCHEMIC CARDIAC MALFUNCTION			
(57) Abstract <p>The present invention is based on the unexpected discoveries that hypothalamic inhibitory factor (HIF) has a positive inotropic effect in a whole organ preparation, such as an isolated perfused heart, and that HIF, to a greater extent than ouabain, dilates the coronary arteries in the isolated perfused heart as manifested in increased coronary flow in hearts treated with HIF. The present invention relates to a method for increasing coronary perfusion in a mammalian host comprising administering to the host in need thereof an effective amount of hypothalamic inhibitory factor (HIF). The invention also relates to a method for producing an increased coronary vasodilatory effect in a mammalian host comprising administering to a mammalian host in need thereof an effective amount of HIF. The method of the present invention can be used to prevent and/or treat ischemic cardiac malfunction and coronary artery restenosis.</p>			

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USE OF HYPOTHALMIC INHIBITORY FACTOR IN THE TREATMENT OF ISCHEMIC CARDIAC MALFUNCTION

BACKGROUND

While heart attack and stroke now cause fewer deaths than previously, deaths due to heart failure (inability of 5 the heart to deliver adequate blood supply to vital organs via the systemic circulation) have increased markedly (82.5%) between 1979 and 1992. In 1997, heart failure is expected to cause at least 250,000 deaths in the United States. More than 400,000 new cases of heart failure are 10 expected to be diagnosed in 1997. In all, it is estimated that more than 4 million Americans are presently living with this dangerous condition.

Sixty to seventy percent of these cases of heart failure are due to ischemic causes, i.e., insufficient 15 delivery of blood flow and vital oxygen via the coronary circulation to the cardiac muscle. This can be due to mechanical obstruction of the coronary arteries, for example by atherosclerotic deposits, to spasm (temporary occlusion) of the coronary arteries, or to restenosis of 20 coronary arteries that have been previously opened by angioplasty (balloon catheter dilation).

Certain segments of the general population are particularly prone to heart failure. Heart attack 25 survivors have a four to six times greater risk of having an additional heart attack. Patients with angina (ischemic coronary pain), diabetes mellitus, or uncontrolled high blood pressure have a two-fold risk of developing heart failure compared to the general population.

Current treatments of heart failure include agents 30 which dilate, and thus, relax blood vessels reducing

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arterial resistance against which the heart must work. Converting enzyme inhibitors such as captopril, enalapril and lisinopril are examples of such arterial vasodilators. Side effects such as rash, persistent cough, excessive 5 lowering of blood pressure and adverse effects on kidney function limit the use of these agents in therapy. Other drugs which work by directly dilating blood vessels (arteries), such as hydralazine, prazocin and doxazocin also reduce the resistance against which the failing heart 10 must pump, but these drugs too can cause excessive lowering of blood pressure, as well as reflex tachycardia (increased heart rate) which increases cardiac work and oxygen demand by the heart muscle already deprived of an adequate oxygen supply in the case of ischemic heart failure.

15 Because of their positive inotropic effect, cardiac glycosides (e.g., digitalis, ouabain) have been considered unrivaled in value for the treatment of heart failure. Positive inotropic effect generally refers to the enhancement of the contractility of the cardiac cells in a 20 dose-dependent manner. Cardiac glycosides are most frequently used therapeutically to increase the adequacy of the circulation in patients with congestive heart failure and to slow the ventricular rate in the presence of atrial fibrillation.

25 However, cardiac glycosides have narrow therapeutic indices and their use is frequently accompanied by toxic effects that can be severe or lethal. The most important toxic effects, in terms of risk to the patient, are those that involve the heart (e.g., abnormalities of cardiac 30 rhythm and disturbances of atrio-ventricular conduction). Gastrointestinal disorders, neurological effects, anorexia,

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blurred vision, nausea and vomiting are other common cardiac glycoside-induced reactions.

In the case of ischemic heart disease, other classes of compounds have been used to improve blood flow to the failing myocardium through dilation of the coronary arteries. Examples of these agents include nitrates, such as isosorbide dinitrate, and calcium channel blockers such as diltiazem, nifedipine and verapamil.

Nitrates and calcium channel blockers do not have direct effects on the contractile mechanism of the cardiac muscle cells. That is, they are not positive inotropic agents. Digitalis and ouabain, on the other hand, are strong inotropes, but do not have vasoactive (dilatory) effects on the coronary circulation. Hypothalamic inhibitory factor (HIF) has also been shown to produce a positive inotropic effect on cardiac muscle cells (U.S. patent application No. 08/338,264, filed November 10, 1994). However, HIF was not shown to have vasoactive (dilatory) effects on the coronary circulation.

A single compound which has both inotropic effects and coronary vasodilatory effects would be highly desirable as an agent to treat ischemic heart failure since both abnormalities are present and each contributes to the morbidity of the condition. To this point, such a compound has not been identified or made available pharmaceutically for the treatment of ischemic heart failure.

SUMMARY OF THE INVENTION

The present invention relates to a method for increasing coronary perfusion in a mammalian host comprising administering to the host in need thereof an

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effective amount of hypothalamic inhibitory factor (HIF). The invention also relates to a method for producing an increased coronary vasodilatory effect in a mammalian host comprising administering to a mammalian host in need thereof an effective amount of HIF.

5 In a particular embodiment, the invention pertains to a method for preventing or treating ischemic cardiac malfunction comprising administering to a mammalian host in need thereof an effective amount of HIF. The invention 10 also relates to a method for preventing or treating coronary artery restenosis comprising administering to a mammalian host in need thereof an effective amount of HIF. Also encompassed by the present invention is a method for 15 treating stenosis of a coronary artery in a mammalian host comprising administering to the host in need thereof an effective amount of HIF. Stenosis of a coronary artery can be due to, for example, atherosclerosis and/or coronary artery restenosis following the intervention of mechanical dilatation or certain bypass graft operations.

20 HIF slows heart rate in spontaneously beating cardiac myocytes, further reducing cardiac work and thereby diminishing oxygen requirement. The invention provides a single compound, HIF, which has the combined properties of producing a positive inotropic effect and enhancing 25 coronary flow, and which can be used prophylactically and/or therapeutically to treat the identified conditions associated with heart failure.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the unexpected 30 discoveries that hypothalamic inhibitory factor (HIF) has a

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positive inotropic effect in a whole organ preparation, such as an isolated perfused heart, and that HIF, to a greater extent than ouabain, dilates the coronary arteries in the isolated perfused heart as manifested in increased 5 coronary flow in hearts treated with HIF. As defined herein "coronary flow" and/ or "coronary perfusion" refers to milliliters per minute of physiologic buffer perfusing the coronary arteries. As demonstrated herein HIF causes both enhanced contractility and simultaneous increase in 10 coronary perfusion when administered to an intact heart. The present invention relates to methods for increasing coronary perfusion in a mammalian host comprising administering to the host in need thereof an effective amount of HIF. The present invention also relates to a 15 method of producing a positive inotropic effect and an increased vasodilatory effect in a mammalian host comprising administering to the host in need thereof an effective amount of HIF. The mammalian host can be any mammal which is in need of increased coronary perfusion, 20 and includes, for example, human, canine, feline, bovine and murine hosts.

HIF is an endogenous inhibitor of $\text{Na}^+ \text{-K}^+$ -ATPase, which has been isolated from bovine hypothalamus and human plasma, and structurally characterized as an isomer of the 25 plant cardiac glycoside, ouabain (Tymiak, A.A., et al., Proc. Natl. Acad. Sci., USA, 90:8189-8193 (1993); Zhao, N., et al., Biochemistry, 34:9893-9896 (1995)). HIF for use in the present invention can be obtained by purifying HIF from natural sources or chemically synthesizing HIF. In a 30 preferred embodiment, purified HIF is used. Purified HIF refers to HIF which is substantially free of or isolated

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from other tissue or fluid protein components and contaminants. Various procedures may be used to purify HIF from natural sources. For example, as described in U.S. patent application No. 08/338,264, filed November 10, 1994, 5 which is incorporated by reference, HIF has been purified to homogeneity using an affinity chromatography method in which purified renal Na⁺, K⁺-ATPase (e.g. isolated from a canine) is coupled to paramagnetic particles through a glutaraldehyde bridge (Tymiak, A.A., et al. *Proc. Natl. Acad. Sci., USA*, 90:8189-8193 (1993)). The enzyme immobilizes bound HIF in the presence of Mg⁺⁺ and inorganic phosphorous with high affinity, and after washing away contaminating materials, HIF is eluted from the affinity column by chelating Mg⁺⁺ with EDTA. A subsequent HPLC step 10 results in purification of HIF (Tymiak, A.A., et al. *Proc. Natl. Acad. Sci., USA*, 90:8189-8193 (1993)). HIF has also been purified to homogeneity using an immunoaffinity chromatography method in which an antibody which binds to HIF is coupled to the resin of an immunoaffinity column. 15 20 The antibody recognizing HIF immobilizes bound HIF with high affinity, and after washing away contaminating materials, HIF is eluted from the affinity column. A subsequent HPLC step can be used to further purify HIF. See U.S. patent application No. _____, filed May 30, 25 1997, Attorney Docket No. BION97-01, which is incorporated by reference. It should be noted that pharmaceutically acceptable salts of HIF are also contemplated. Suitable salts include those well known to those of skill in the art. As defined herein, an "effective amount" is an amount 30 sufficient, when administered to the host, to result in an increased coronary vasodilatory effect and, preferably, a

positive inotropic effect also in the mammalian host relative to the inotropic and coronary vasodilatory effects when an effective amount of HIF is not administered. These effects are associated with increased coronary perfusion 5 and can be utilized to treat and/or prevent ischemic cardiac malfunction, coronary artery restenosis and stenosis of a coronary artery in a mammalian host. The amount of HIF used to treat a host will vary depending on a variety of factors, including the size, age, body weight, 10 general health, sex and diet of the host. In addition, an effective amount of HIF will depend on the nature of the disease, and can be determined by standard clinical techniques. The precise dose to be employed will also depend on the route of administration and the seriousness 15 of the cardiac disease or disorder, and should be decided according to the judgement of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 4.5 ug to about 20 ug per kilogram body weight. In one embodiment 20 the dosage is about 4.5 ug per kilogram body weight, in another embodiment the dosage is about 12 ug per kilogram body weight, and in a further embodiment the dosage is about 20 ug per kilogram body weight. Suitable dose ranges for oral administration of HIF are the same as for 25 intravenous administration of HIF, but the HIF dosage is administered orally over several days. Effective doses may be extrapolated from dose response curves derived from *in vitro* or animal test models.

The HIF of the present invention can be administered 30 prophylactically to a host as a method of preventing the conditions described herein. Alternatively, HIF can be

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administered therapeutically to a host as a method of treating an existing disease and/or condition in the host, and can result in amelioration or elimination of the disease and/or condition.

5 The formulation and route of delivery of HIF to the host can be accomplished in a variety of ways. Routes of administration include intradermal, transdermal (e.g. slow release polymers), intramuscular, intraperitoneal, intravenous, intracardiac, subcutaneous, oral, epidural, 10 and intranasal routes. Any other convenient route of administration can be used, for example, infusion or bolus injection, or absorption through epithelial or mucocutaneous linings. The HIF can be administered together with other components or biologically active 15 agents, such as pharmaceutically acceptable surfactants (e.g., glycerides), carriers (e.g., saline, buffered saline, dextrose, water, glycerol, ethanol), excipients (e.g., lactose), diluents and vehicles and combinations thereof. The formulation should suit the mode of 20 administration.

 The composition can also include, if desired, minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained 25 release formulation or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium 30 saccharine, cellulose, magnesium carbonate, etc.

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In a preferred embodiment, the HIF is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to the mammalian host (e.g., a human). For example, HIF for 5 intravenous administration can be a solution in sterile isotonic aqueous buffer. Where necessary, the HIF composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of injection. Generally, the ingredients are supplied either separately 10 or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampule of 15 sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration. Thus, the invention also relates to the use of HIF in the manufacture of medicaments for the treatment and/or prevention of ischemic cardiac malfunction, coronary artery 20 restenosis and/or stenosis of a coronary artery.

The invention can be used to prevent and/or treat a patient with ischemic heart failure, which is due, for example, to stenosis of the coronary artery. It is estimated that four million Americans suffer from heart 25 failure, and that 60-70% of these cases (2.4-2.8 million) are on the basis of ischemic disease. The combined advantages over digitalis and ouabain of less toxicity and enhanced coronary perfusion makes the invention a therapeutically desirable substitute for digitalis in these 30 patients.

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The invention also relates to methods wherein HIF is administered to prevent and/or treat coronary artery restenosis in patients who have undergone coronary artery angioplasty or certain kinds of coronary artery bypass 5 surgery for conditions such as ischemic heart disease.

HIF does not manifest the same toxicity profile as the cardiac glycosides. Therefore, prevention and/or treatment of conditions associated with heart failure, such as ischemic cardiac failure and coronary artery restenosis, 10 with HIF can be accomplished with less risk of toxicity to the patient, and both abnormal muscular contractility and inadequate flow of blood and oxygen to the ischemic myocardium can be concurrently treated and/or prevented with a single compound.

15 This invention is illustrated further by the following exemplification, which is not to be construed as limiting in any way.

EXEMPLIFICATION

Measurement of positive inotropic effects and coronary flow

20 Guinea pigs were anesthetized with 65-100 mg pentobarbital in the peritoneal cavity followed by anticoagulation using 200 units of heparin via femoral vein. Sternotomy was performed, the beating heart removed and placed in a bowl containing room temperature saline. 25 The aorta was trimmed and cannulated onto the perfusion apparatus. Retrograde perfusion of Kreb's solution (37°C) at 70 mm Hg bubbled with 95% O₂/5% CO₂ from a 3 liter reservoir was begun within one minute of cardectomy. A micro flexible temperature probe was inserted in the open

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pulmonary artery allowing coronary effluent to drain. The left atrial appendage was incised, the left ventricle vented, and a water-filled balloon-tipped catheter inserted into the left ventricle chamber. Left ventricular systolic 5 pressure (LVP), left ventricular end diastolic pressure (LVEDP), maximal rate of rise of left ventricular pressure (dP/dT), and aortic root pressure (AOP) were recorded on a precalibrated multichannel dynograph. Coronary flow (CF) is measured by timed volumetric collection. The heart was 10 paced (HR) at 272 BPM by way of electrodes attached to the right atrium. After 30 minutes of perfusion, coronary flow was then diverted into a recirculating perfusion circuit primed with 60 cc of Kreb's solution.

The circuit was started with the coronary effluent 15 draining into a reservoir. The effluent was pumped out the reservoir through a 5 micron filter that passed through a low volume silastic oxygenator and up a pressure column. The oxygenated effluent flowed back to the aorta or out an overflow column that drained to the reservoir. Pump output 20 was kept higher than the coronary flow allowing a constant aortic root pressure. Heart temperature (HT), pH, O₂ and CO₂ of the perfusate were held constant by water jacketing the oxygenator and perfusion circuit and no additional Kreb's solution needed to be added during the recirculation 25 intervals. During the first ten minutes of recirculating, the intraventricular balloon volume was adjusted to reach an LVEDP of 10 mm Hg. The heart was switched back to non-recirculation for ten minutes while the recirculating circuit was emptied and refilled with fresh Kreb's 30 solution. Two additional recirculation non-recirculation cycles were carried out with LVEDP adjusted and parameters

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measured. LVP, LVEDP, dP/dT, AOP, HR, HT and CF were recorded at 5 minute intervals for 15 minutes. Then a coded aliquot of ouabain, HIF or placebo was added to the recirculating Kreb's solution. Parameters continued to be measured at 5 minute intervals for 30 additional minutes.

Results

HIF has been previously shown to produce positive inotropic effects in isolated, spontaneously beating myocytes in culture, with greater potency and a different toxic profile from ouabain (Hallaq, H. and Haupert, G., Jr., *Proc. Natl. Acad. Sci., USA*, 86:10080-10084 (1989)). As shown herein, HIF also produces a positive inotropic effect in an intact, whole organ preparation. 20 μ g HIF was prepared to homogeneity as confirmed by mass spectral measurement, for infusion into an isolated, perfused guinea pig heart preparation according to the Langendorf methodology (Hendren, W.G., et al., *J. Thor. Cardiovasc. Surg.*, 94:614-625 (1987)). This methodology allows continuous measurement of left ventricular pressure generation, the first derivative of the pressure measurement (dP/dT), end diastolic pressure and coronary flow. Pilot studies with ouabain were carried out to determine the minimal consistently effective dose to produce an enhanced inotropic effect. For both ouabain and HIF, this amount was 4 μ g of pure inhibitor administered to a perfusion volume of 60 ml Krebs-Heinsleit buffer (final concentration 1×10^{-7} M). Coded samples were prepared of HIF, ouabain and placebo (vehicle), and administered randomly to the preparation. The one conducting the experiments was blinded to the samples.

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Results are shown in the Table. The Table shows the effects of HIF, ouabain and placebo on performance characteristics and coronary flow in isolated, perfused guinea pig heart. Both HIF and ouabain showed significant 5 increases in left ventricular pressure (lvp) and rate of development of pressure (dP/dT) compared to the preadministration control period, indicating positive inotropic response. Placebo produced no change. HIF and ouabain also produced a statistically significant increase 10 in coronary flow, but the percent increase from baseline for HIF surprisingly was two-fold greater than the increase produced by ouabain (Table). Thus, the mammalian-derived HIF was a significantly more potent coronary vasodilator than the plant-derived ouabain. HIF produced a 15 statistically significant increase in coronary flow and is less toxic than ouabain.

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TABLE 1: Effects of HIF, ouabain and placebo on performance and perfusion characteristics in isolated, perfused guinea pig heart (LVP, left ventricular pressure; dP/dt, rate of development of pressure)

GROUPS	LVP	LVP post intervention	dPdt	dP/dt post intervention	End Dia pressure	EDP post intervention	coronary flow cc/min	CF post intervention				
ouabain n=9	73.82	90.44^*	22.5	2326	2856^*	22.7	7.66	8.01	4.6	25.84	27.77*	7.5
placebo n=8	67.17	68.29	1.7	2104	2088	-0.7	7.77	9.19	18.2	25.89	25.09	-3.1
HIF n=9	70.54	89.91^*	27.4	2292	2884^*	25.8	7.6	7.77	2.1	24.37	27.82*	14.1

^ P<0.01 intervention vs placebo

* P<0.01 pre intervention vs post intervention

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EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific embodiments of the 5 invention described herein. Such equivalents are intended to be encompassed in the scope of the following claims.

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CLAIMS

We claim:

1. A method for increasing coronary perfusion in a mammalian host comprising administering to the host in need thereof an effective amount of hypothalamic inhibitory factor.
5
2. The method of Claim 1 wherein the mammalian host is a human.
3. The method of Claim 1 wherein the hypothalamic inhibitory factor is administered intravenously.
10
4. A method for producing an increased coronary vasodilatory effect in a mammalian host comprising administering to a mammalian host in need thereof an effective amount of hypothalamic inhibitory factor.
- 15 5. The method of Claim 4 wherein the mammalian host is a human.
6. The method of Claim 4 wherein the hypothalamic inhibitory factor is administered intravenously.
7. A method for preventing or treating ischemic cardiac
20 malfunction comprising administering to a mammalian host in need thereof an effective amount of hypothalamic inhibitory factor.

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8. The method of Claim 7 wherein the mammalian host is a human.
9. The method of Claim 7 wherein the ischemic cardiac malfunction is due to stenosis of the coronary artery.
- 5 10. The method of Claim 7 wherein the hypothalamic inhibitory factor is administered intravenously.
11. A method for preventing or treating coronary artery restenosis comprising administering to a mammalian host in need thereof an effective amount of hypothalamic inhibitory factor.
- 10 12. The method of Claim 11 wherein the mammalian host is a human.
13. The method of Claim 11 wherein the hypothalamic inhibitory factor is administered intravenously.
- 15 14. A method for treating stenosis of a coronary artery in a mammalian host comprising administering to the host in need thereof an effective amount of hypothalamic inhibitory factor.
- 20 15. The method of Claim 14 wherein stenosis of the coronary artery is due to coronary artery restenosis.
16. The method of Claim 14 herein the mammalian host is a human.

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17. The method of Claim 14 wherein the hypothalamic inhibitory factor is administered intravenously.

INTERNATIONAL SEARCH REPORT

Int. Application No

PCT/US 98/10890

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 433 074 A (GEN HOSPITAL CORP) 19 June 1991 cited in the application see abstract; claims 1-3 ---	7-10
A	N. ZHAO ET AL.: "NA,K-ATPase INHIBITORS FROM BOVINE HYPOTHALAMUS AND HUMAN PLASMA ARE DIFFERENT FROM OUABAIN: NANOGRAM SCALE CD STRUCTURAL ANALYSIS" BIOCHEMISTRY, vol. 34, no. 31, 1995, pages 9893-9896, XP002081420 cited in the application see the whole document ---	1-17 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

Int'l Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>TYMIAK A A ET AL: "PHYSICOCHEMICAL CHARACTERIZATION OF A OUABAIN ISOMER ISOLATED FROM BOVINE HYPOTHALAMUS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 90, no. 17, 1 September 1993, pages 8189-8193, XP002074993 cited in the application see the whole document</p> <p>---</p>	1-17
A	<p>R. KOHN ET AL.: "ENDOGENOUS DIGITALIS-LIKE FACTOR IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION" COR ET VASA, vol. 34, no. 3, 1992, pages 227-237, XP002081545 see the whole document</p> <p>---</p>	1-17
A	<p>P. DELVA ET AL.: "INCREASE IN PLASMA DIGITALIS-LIKE ACTIVITY DURING PERCUTANEOUS TRANSLUMINAL CORONARY ANGIOPLASTY IN PATIENTS WITH CORONARY STENOSIS" LIFE SCIENCE, vol. 47, no. 5, 1990, pages 385-389, XP002081546 see the whole document</p> <p>-----</p>	1-17

INTERNATIONAL SEARCH REPORT

...international application No.

PCT/US 98/10890

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-17 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking(Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/10890

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